FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

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INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/04310	22 June 1999	22 June 1998

TITLE OF INVENTION

IMPROVEMENT OF BINDING ASSAYS BY MULTIEPITOPE ANALYSIS AND BY COMBINING ANTIGEN AND ANTIBODY DETERMINATION

APPLICANT(S) FOR DO/EO/US

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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409)
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment. (to follow)
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

General Appointment of Representative for U.S. Patent and Trademark Office Matters (1pp); and Return postcard.

U.S APPLICATION NO. (IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/720006	PCT/EP99/04310	RDID00115US

21. The following fees are submitted:	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO **$840.00**	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$690.00**	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	- 20 =	0	x $18.00	**$0.00**
Independent claims	- 3 =	0	x $78.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**

TOTAL OF ABOVE CALCULATIONS =	**$860.00**
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).** ☐	**$0.00**
SUBTOTAL =	**$860.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)) +	**$0.00**
TOTAL NATIONAL FEE =	**$860.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐	**$40.00**
TOTAL FEES ENCLOSED =	**$900.00**
	Amount to be: refunded $
	charged $

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **50-0877** in the amount of **$900.00** to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **50-0877.** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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19 December 2000
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Improvement of binding assays by multiepitope analysis and by combining antigen and antibody determination

Description

The invention concerns a method for the detection of one or several analytes in a sample in which the analyte is detected using different reagents that are capable of binding to the analyte. Furthermore the invention concerns a solid phase for the detection of an analyte, the solid phase comprising a non-porous support and spatially separate test areas, whereby each test area contains different reagents. The invention also concerns a method for the simultaneous determination of an antigen and of an antibody which is specifically directed against this antigen as well as a solid phase for carrying out this method.

Many analytes can be determined by immunological detection methods. Such immunological detection methods utilize the ability of analytes to specifically bind to certain reagents such as antigen-antibody interactions. Immunological determinations can be carried out in a number of basic test formats such as the sandwich test format, the indirect test format, the back titration format or the bridge format.

A reliable detection of infectious diseases e.g. an infection with viruses such as HIV, HBV or HCV, is of particular interest to enable diagnosis of the disease in the infected persons as early as possible. Immunological determinations of antibodies against HIV, HBV or HCV are generally carried out in an indirect test

format or by means of the bridge format. The antibody is usually detected using mixtures of various proteins or peptides which include epitopes from the core and envelope region of the pathogen. This mixture is immobilized on a support i.e. a solid phase. Since a classification as HIV-positive has major implications for the individual and false-positive results can have fatal consequences, all positive results obtained in routine tests with this immunological determination must at present be checked in a confirmation test. Usually a Western blot is used as a confirmation test in which the individual protein components of a viral lysate are blotted on a porous support. However, in the case of HCV it is very difficult to culture the virus. Hence in this case a Western blot with a viral lysate is not used as a confirmation test but rather a RIBA (recombinant immunoblot assay) is carried out which is an immunodot-blot using recombinant proteins or peptides as test reagents.

A major disadvantage of the currently used routine tests is that mixtures of 5 to 10 or more antigens are used for the detection depending on the analyte. Although the routine tests are being continuously improved, it has not yet been possible to completely omit a confirmation test. For example a mixture of ca. five different antigens is used in the Enzymun® HIV test (Boehringer Mannheim) which are biotinylated as well as labelled with digoxigenin for the detection. Although the test functions well, the use of antigen mixtures comprising such a large number of different antigens means that the individual antigens immobilized or bound on the solid phase can no longer be present at an optimal concentration for the test. With such a mixture of many components, the binding capacity of the solid phase is

no longer adequate to bind all antigens at an optimal concentration. Furthermore when coating a test area, the use of an antigen mixture results in competition between the various antigens for the binding sites on the solid phase and the different proportions lead to different diffusion rates and to different steric effects. In a direct coating hydrophobic antigens are for example preferentially bound to the plastic surface whereas more hydrophilic antigens are at the same time displaced. This leads, on the one hand, to poorly reproducible results and, on the other hand, the concentration of certain antigen epitopes becomes so low that a significant detection is no longer possible.

A further disadvantage of using antigen mixtures in routine tests is that the risk of an increased unspecific binding is considerably increased by the mixture of different antigens which in turn can lead to an increase of false-positive results. As a consequence the cut-off limit of the previously used routine tests has to be set at a relatively high level and thus sensitivity is lost. The number of false-positive results due to unspecific binding increases considerably especially in the Western blot due to foreign proteins present in the viral lysate and consequently at least two reactive bands are required for a positive result.

It has been attempted to further improve the sensitivity of these detection methods. EP 0 461 462 A1 describes an immunoassay for the detection of viral antibodies with the aid of an indirect test concept. In the immunodot-blot described in EP 0 461 462 A1 purified recombinant proteins are applied instead of a conventional viral lysate in discrete test areas on a porous support to obtain a test format which is more sensitive than a

Western blot due to the use of purified proteins.

EP 0 627 625 A1 concerns a method for the detection of viral antibodies in a sample by means of a bridge concept. This method is also a RIBA (recombinant immunoblot assay) in which several antigens are applied spatially separated on a solid phase made of a porous material; it is pointed out that it is necessary to use a solid phase made of porous material.

EP 0 445 423 A2 concerns a method for the detection of HCV antibodies with the aid of several epitopes of a HCV antigen. In EP 0 445 423 A2 an immunodot assay is also described for the antibody determination, a higher sensitivity being achieved by the use of certain, improved antigens.

However, the defined application of a predetermined amount of reagent is difficult to accomplish in these methods described in the prior art due to the use of a porous support. In particular there is a risk that the individually applied test spots will coalesce. These disadvantages become more serious the smaller the applied spots become and thus these methods are not suitable especially for miniaturized test systems. In addition it is difficult to automate the handling of paper strips which are thus inconceivable as a routine test.

One object of the invention was therefore to provide a method which can at least partially eliminate the disadvantages of the prior art.

This object is achieved according to the invention by a

method for the detection of an analyte in a sample comprising the steps:

(a) providing a solid phase which comprises a non-porous support and at least two spatially separate test areas, the test areas each containing different immobilized analyte-specific receptors,

(b) contacting the sample with the solid phase and with at least one free analyte-specific receptor which carries a signal generating group or is capable of binding to a signal generating group, and

(c) detecting the presence or/and the amount of the analyte by determining the signal generating group on the test areas.

The immobilized analyte-specific receptor can be bound directly or indirectly to the solid phase via one or several receptors. It can for example be bound by means of adsorptive or covalent interactions, but preferably by specific high-affinity interactions e.g. streptavidin or avidin/biotin or antibody-antigen interactions.

The free analyte-specific receptor can itself carry a signal generating group or it can bind to a signal generating group. In this case the detection reagent is composed of several components.

The analyte can be a homogeneous or a heterogeneous population e.g. a heterogeneous antibody population, an antigen mixture or a mixture of antigens and antibodies that may be different, the antigens and antibodies being derived from or induced by one or several pathogens. In the case of heterogeneous analyte populations, the individual test areas bind a partial population of the analyte to be determined. Each of the analyte-specific receptors immobilized on a test area is different i.e.

they bind, according to the invention, preferably to different epitopes of a homogeneous analyte such as an antigen, to different analyte subtypes such as antigen subtypes or/and to different analyte types such as different antigens or/and antibodies.

It was surprisingly found that the sensitivity of detection tests such as antibody tests can be considerably improved by the use of panel tests in which the various reagents, e.g. various antigens, are applied as individual spots i.e. are applied individually to separate test areas. The multiepitope analysis according to the invention i.e. the simultaneous separate detection of several partial populations of an analyte or pathogen such as HIV can considerably improve the sensitivity and reliability of detection tests.

If a positive test result is obtained on one or several, and in some cases on at least two test areas, this is assessed as indicating the presence of the analyte in the sample.

The use of a non-porous support according to the invention enables the reagents to be applied in defined areas. This is of particular importance for miniaturized test formats. Accordingly the test areas preferably have a diameter of 0.01 to 1 mm, more preferably of 0.1 to 0.5 mm and most preferably of 0.1 to 0.2 mm.

Solid phases with several test areas are preferably used which are also referred to as array systems. Such array systems are for example described in Ekins and Chu (Clin. Chem. 37 (1995) 1955-1967) and in the US patents 5,432,099, 5,516,635 and 5,126,276.

The solid phase used according to the invention comprises a non-porous support that can be used for detection methods. This non-porous support can be composed of any non-porous material. The support preferably has a plastic, glass, metal or metal oxide surface. The support particularly preferably has a polystyrene surface. Spatially discrete regions (test areas) are arranged on this support. Reagents such as immobilized solid phase receptors are applied to these test areas. The reagents are immobilized on the test areas by known methods e.g. by direct adsorptive binding, by covalent coupling or by coupling by means of high-affinity binding pairs e.g. streptavidin/biotin, antigen/antibody or sugar/lectin.

It is particularly advantageous to load the spatially separate test areas separately with different reagents. The individual application of the various test areas enables an optimal solid phase concentration and optimal coating conditions, e.g. in the form of special buffer compositions, to be selected for each reagent, for example for each individual antigen. As a result it is possible to coat each individual analyte-specific receptor, e.g. each individual antigen, up to the maximum binding capacity of the area whereas in the previously known tests each receptor e.g. each antigen was bound utilizing only part of the available binding capacity. Furthermore as a result of the separate application of the various reagents, there is no competition between the individual reagents, for example the antigens, for the binding sites on the solid phase. Accordingly it is preferred to bind only one reagent which can bind specifically to the analyte to be determined per test area such that each test area contains only a single type of an immobilized, analyte-

specific receptor. This reagent can optionally be diluted by inert diluent molecules in order to form an optimal homogeneous binding phase. Inert diluent molecules are molecules which bind to the solid phase but do not interact with the analyte or other sample components. Suitable diluent molecules are described for example in WO 92/10757 and in EP 0 664 452 A2.

It was found that unspecific binding is considerably reduced in test areas on which only a single reagent capable of binding to the analyte e.g. an antigen is bound. Thus for example when different antigens are applied as individual spots, no measurable unspecific binding is observed, whereas a test spot on which a mixture of several antigens has been applied exhibits a clearly measurable unspecific binding.

In the method according to the invention the analyte is detected in a known manner by the use of suitable marker groups e.g. fluorescent marker groups, chemiluminescent marker groups, radioactive labels, enzyme labels, coloured labels and sol particles. Alternatively, if the solid phase is suitable, the interaction of components of the detection medium with the test areas can also be detected by determining the layer thickness of the respective areas e.g. by plasmon resonance spectroscopy.

Furthermore in order to distinguish between the discrete test areas and inert regions of the solid phase, they can contain a detectable and analyte-unspecific marker group which can be detected concurrently with the analyte-specific coating group and does not interfere with it. An example of such an analyte-unspecific marker group is a fluorescent marker group which fluoresces at

a wavelength that is different from the fluorescent wavelength of an analyte-specific marker group. The analyte-unspecific marker group is preferably immobilized, like the solid phase receptor, by means of a high affinity binding pair e.g. streptavidin/biotin.

The sensitivity can be further increased by using a universal detection reagent. Before this, a separate detection reagent can be used for each analyte molecule which binds to the analyte molecule and carries a label such as an enzyme, a fluorescent label or fluorescent latex particles. However, a combination of several labelled detection reagents often results in a very high concentration of labels and consequently the unspecific binding increases strongly. This problem can be resolved by using a universal detection reagent. According to the invention, fluorescent-labelled latex particles are preferably used as the universal detection reagent. In this case an analyte-specific first receptor which itself carries no signal generating group is used to specifically bind the analyte molecule. A universal second labelled receptor, i.e. a receptor which binds analyte-independently to several, preferably to all first receptors, binds to this analyte-specific first receptor. The second receptor can be coupled adsorptively or covalently via functional groups to the marker group or by means of high affinity binding pairs e.g. streptavidin/biotin, antigen/antibody or sugar/lectin. It is preferable to use the well-known dig/anti-dig system.

A further disadvantage of the bridge tests which are for example carried out as a one step reaction, is that the solid phase receptor (e.g. biotinylated HIV-gp41) and the free detection receptor (e.g. digoxigenylated HIV-

gp41) have to be provided in a 1:1 ratio to obtain an optimal signal. This is disadvantageous since the concentration of the individual solid phase receptors is often suboptimal due to the limited binding capacity of the solid phase and may thus also not be favourable for the detection receptor.

The method according to the invention allows the solid phase receptors to be already bound to the solid phase at an optimal concentration. Furthermore the detection receptor can also be supplied at an optimal concentration since receptor conjugates with digoxigenin or biotin have a negligible tendency for unspecific binding in contrast to enzyme-labelled receptors. One can use these reagents in excess so that imprecisions in the addition of the receptor do not have an effect on test precision.

Specific binding of the analyte to be determined to the reagent, e.g. a solid phase receptor, immobilized on the test area enables the presence or/and the amount of the analyte in the sample to be determined. The combined evaluation of different test areas which each contain different reagents which can bind specifically to the analyte, considerably improves the sensitivity of the detection method especially by reducing false-positive results and by enabling the unequivocal recognition of true positive results. The method according to the invention is of particular interest for the detection or elimination of unspecific binding in qualitative tests where high demands are made on the specificity, such as tests for infections (e.g. HIV).

The use of arrays according to the invention i.e. solid

phases which comprise at least two, more preferably at least three, most preferably at least five and up to one thousand and more preferably up to one hundred spatially separate test areas, enables at least one of these test areas to be designed such that it represents a control area. Consequently the method according to the invention preferably comprises the use of a solid phase which additionally contains at least one, more preferably two and most preferably at least five control areas. The integration of control spots in the solid phase enable false results caused by interferences to be easily and rapidly detected. In addition to the specific test areas, it is also possible to measure a sample-specific background and thus to define a sample-specific cut-off. The use of a test array and the use of control spots allows the cut-off limit to be lowered. The cut-off value is a threshold value which is used in test procedures to differentiate between positive and negative values. Such a cut-off value is particularly important for test procedures that relate to infectious diseases. The method according to the invention allows a positive-negative differentiation to be made with a considerably reduced probability of error.

If several test areas are used which each allow the determination of different analyte molecules, it has often proven to be expedient to define a test area specific cut-off value in order to obtain an increased test specificity (i.e. a correct differentiation between positive and negative values) while retaining the sensitivity.

The method according to the invention can be used for any detection methods e.g. for immunoassays, nucleic acid hybridization assays, sugar-lectin assays and

similar methods. The method according to the invention is also basically suitable for the detection of any analytes in a sample. The analyte is particularly preferably detected by means of specific interactions with one or several reagents capable of binding to the analyte i.e. receptors which are preferably selected from proteins, peptides, antibodies, antigens, haptens and nucleic acids.

Whereas a major advantage of the method according to the invention is primarily to improve the sensitivity of a test for a single analyte, it is also possible to determine several analytes simultaneously with high sensitivity when the test areas are selected appropriately.

A further subject matter of the present invention is a solid phase for the detection of an analyte in a sample which is characterized in that it comprises a non-porous carrier and at least two spatially separate test areas, the test areas each containing different reagents which are capable of binding specifically to the analyte to be determined.

The test areas preferably each contain different reagents which bind to different epitopes or/and subtypes of an analyte or/and to different analyte types.

Miniaturized test formats are preferably used in order to accommodate the largest possible number of test areas on a solid phase. The distance between the individual test areas is selected such that the applied reagents cannot coalesce. This can usually be prevented when the

distance between the edges of the test areas is 0.05 to 5 mm. There is preferably an inert surface between the test areas which can neither bind to the analyte nor to other sample components.

The solid phase according to the invention can be used in any detection methods e.g. in immunoassays, nucleic acid hybridization assays, sugar-lectin assays and such like. It is preferably used in an immunoassay for the detection of antibodies or/and antigens.

The invention additionally concerns a test kit for the detection of an analyte in a sample which comprises a solid phase according to the invention and labelled detection reagents. Labelled detection reagents are known to a person skilled in the art and generally comprise a marker group and a specific bindable group which allows the analyte to be detected. Suitable marker groups are for example fluorescent, chemiluminescent, enzyme, radioactive or particle (sol) marker groups. The specifically bindable group can for example be a group that can bind to the analyte complex that is formed or in the case of competitive test formats, with other components of the detection system. The test kit preferably contains a universal conjugate as a detection reagent, especially fluorescent labelled latex particles which is able to bind to the detection receptors that are specific for the analyte.

A further problem of conventional routine tests is that it is not possible to simultaneously determine an antigen and an antibody that is directed specifically against this antigen in one measurement. For this reason the antigen p24 and antibodies against other HIV

antigens are for example determined simultaneously in so-called HIV combination tests. In such a test it is then only possible to determine antibodies against other HIV antigens such as gp41 or gp120, whereas it is not possible to determine antibodies against p24.

US-PS-5,627,026 describes a method for the detection of an antibody and an antigen in a biological sample. Thus for example an assay is described for the determination of the FeLV antigen and the FIV antibody. However, according to the method of US-PS-5,627,026, when an antigen is determined, it is only possible to detect antibodies in the same test which are directed against other antigens.

Hence a further object of the present invention was to provide a method for the simultaneous determination of an antigen and of an antibody that is specifically directed against this antigen in a sample. This object is achieved by a method comprising the steps

(a) providing a solid phase on which an immobilized receptor that can bind to the antigen to be determined is applied in a first test area and an immobilized receptor that can bind to the antibody to be determined is applied in a second test area which is spatially separated therefrom,
(b) contacting the sample with the solid phase and a free analyte-specific receptor which carries a signal generating group or is capable of binding to a signal generating group and
(c) detecting the presence or/and the amount of the antigen and of the antibody by determining the signal generating group on the solid phase.

The antigen is preferably detected using a sandwich test

and the antibody is preferably detected using a bridge concept, a back titration concept or an indirect test format.

The antibody is preferably detected using a back titration. An advantage of this is that when a sandwich test is used to simultaneously detect the antigen, no interference between detection molecules can take place since in this case the same detection reagent can be used to detect the antigen and to detect the antibody. In the case of a sandwich test for the detection of an antigen e.g. HIV-p24, an antibody directed against this antigen is for example immobilized on a test area. A second directly or indirectly labelled antibody directed against the antigen e.g. a digoxigenylated anti-p24 antibody can then be used as the free receptor that is used for the detection. In order to detect the corresponding antibody directed against the antigen e.g. an anti-p24 antibody using a back titration, an antigen that can bind to the antibody, e.g. p24 or a fragment thereof, is immobilized on another test area. The second labelled antibody directed against the antigen, e.g. a digoxigenylated anti-p24 antibody which competes with the analyte that is for example the naturally occurring anti-p24 antibody in the sample for binding to the immobilized antigen is also used as a detection reagent. Thus the same detection reagent such as a digoxigenylated anti-p24 antibody can be used for the preferred simultaneous detection of a p24 antigen and of the anti-p24 antibody which is directed against it.

If the antigen is detected by a sandwich test and the antibody is detected in parallel by a bridge test or an indirect test, special test reagents have to be used to prevent interactions between the detection reagents. In

the case of an indirect test for the detection of an antibody e.g. an anti-p24 antibody, an antigen for which the antibody to be detected is specific, e.g. p24, is for example immobilized on a test area. Then a labelled antibody which recognizes the antibody to be detected but not the immobilized antigen e.g. a digoxigenylated anti-human IgG antibody is used to detect the antibody. When an antigen determination is carried out concurrently in a sandwich format, one or several antibodies, preferably monoclonal antibodies, whose epitope binding sites are known can be used on the detection side. Native or recombinant antigens that contain epitopes capable of binding to the detection antibody cannot be used simultaneously for the antibody determination in an indirect test format or in a bridge format since otherwise an undesired reaction would occur. Instead it is necessary to use predetermined recombinant antigens or peptide antigens without the epitope binding sites to which the detection antibody or antibodies bind.

The multiepitope analysis using array systems according to the invention enables antigen and antibody tests for a particular antigen and for an antibody directed against this particular antigen to be carried out in combination. This procedure enables the diagnostic gap to be closed between the first occurrence of an antigen and the occurrence of antibodies at a later time which exists with the known methods of the prior art and to classify a sample at a very early stage as positive or negative. Usually samples from patients are taken where the sensitivity of a test is determined by the earliest possible detection of positive samples. During an infection the various markers which indicate this infection such as antigens or antibodies directed

against these antigens occur with different time courses.

The multiepitope method according to the invention with array arrangement also enables a specific differentiation to be made between antigen and antibody tests by the arrangement of spatially separate, individual test areas. The advantage of the method according to the invention is seen especially in HIV tests. A preferred example of the method according to the invention is the simultaneous detection of a HIV antigen and antibodies directed against it e.g. of the p24 antigen and the corresponding anti-p24 antibody. In a HIV infection p24 antigens appear first. These can be detected with an antigen test but not with an antibody test. After the appearance of the antigens, antibodies against these antigens are formed. However, in the conventional combination tests it is not possible to combine the p24 antigen test with an anti-p24 antibody test but rather the p24 antigen test is combined with an anti-gp41 antibody test. But since the formation of anti-gp41 antibodies can only take place after formation of anti-p24 antibodies, false-negative results can be obtained with conventional methods in the period until anti-gp41 antibodies are formed. In contrast the method according to the invention is more reliable since anti-p24 antibodies can also be determined.

According to the invention the bindable coating of the first test area in which it is intended to detect the antigen is composed of immobilized antibodies that are specific for epitopes of the antigen to be detected. As a result of the preferred array structure it is possible to apply several antibodies that are specific for different subtypes of the antigen to be detected in

separate test areas. The antibodies are selected according to the antigen to be analysed. When screening for a viral infection, anti-HIV I antibodies, anti-HIV II antibodies, anti-HBV antibodies or/and anti-HCV antibodies are preferably tested. Analogously the bindable coating of the other test areas on which it is intended to detect an antibody preferably contains antigens that are specific for the antibody to be detected. Also in this case it is possible to basically use any antigens that are suitable for the respective test; antigens or epitopes thereof from HIV I, HIV II, HBV or/and HCV are preferably used.

The use of a non-porous solid phase enables particularly good results to be obtained with the method according to the invention. A non-porous solid phase is especially advantageous for applying the test reagents and enables a defined application without the individual test areas merging into one another. In addition the use of non-porous test phases enables a miniaturization of the test format. Miniaturized test formats enable a plurality of test areas to be set up on a single non-porous solid phase.

The binding of an antigen or an antibody to the test areas is preferably detected using labelled antibodies which are directed against the analyte. When the antigen is detected in a sandwich format, a labelled antibody directed against this antigen is used. The same labelled antibody is also used to detect the analyte antibody in a competitive format e.g. a back titration. The spatially separate evaluation of the individual test areas thus enables the detection of antigen as well as the antibody specific for this antigen using a single detection reagent without the two detection methods

interfering with one another. Suitable labelling substances for labelling antibodies are known to a person skilled in the art and include for example fluorescent groups, chemiluminescent groups, radioactive labels, enzyme labels, coloured labels and sol particles. The use of a universal detection reagent is preferred and in particular of fluorescent labelled latex particles which can for example bind to the detection receptors.

Particularly good results are obtained with the method according to the invention when the specifically bindable coatings on the individual test areas are applied separately. This enables the binding capacity of the individual test areas to be optimally utilized and enables the preparation of coatings with an optimal binding capacity. The binding reagents can also be optionally diluted with diluent molecules in order to further improve the binding capability of the coating. Suitable diluent molecules are molecules which do not bind to the analyte to be determined and which also do not interact or bind unspecifically to other sample components which could lead to false-positive results (cf. WO92/10757, EP 0 664 452 A2). The coating in the individual test areas is particularly preferably formed from a single type of molecule capable of specific binding. In this case different reagents capable of binding specifically to the analyte are applied to different test spots. In this manner it is possible to further increase the sensitivity of the method according to the invention.

A further subject matter of the present invention is a solid phase for the simultaneous determination of an antigen and of an antibody directed specifically against

this antigen comprising at least one first test area and at least one second test area which is characterized in that the first test area has a coating that can bind specifically to an antigen and the second test area contains a coating that can bind specifically to an antibody directed against the antigen, whereby the coatings are homogeneous and each contains only a single type of reagent with binding capability. The coatings are applied uniformly to the test areas i.e. they are homogeneous. In addition to the reagent with binding capability, the test areas can also contain inert diluent molecules which can neither interact with the analyte to be detected nor with other sample components.

Whereas it is basically possible to use any support materials, the test areas of the solid phase according to the invention are preferably applied to a non-porous support. The use of non-porous surfaces enables in particular a miniaturization of the test format and the simultaneous determination of a plurality of test areas.

The solid phase according to the invention is particularly suitable for use in an immunoassay for the simultaneous detection of an antigen and of an antibody that is directed specifically against this antigen. In this manner it is possible to further improve the sensitivity and reliability of immunological tests.

The invention also concerns a test kit for the simultaneous determination of an antigen and of an antibody directed specifically against this antigen which comprises the solid phase according to the invention and labelled detection reagents for the detection of bound antigen and antibody on the test

areas. Suitable detection reagents are for example labelled antibodies whereby the label can be selected from the above-mentioned groups.

A further problem of the previously available routine tests is that all the antigens and antibodies necessary for a test, for example a HIV test, are mixed and that a cut-off limit that is optimal for this mixture is determined for the detection method. However, using a common cut-off limit for all parameters means that the cut-off limit is determined and constrained by the unspecific binding of the worst component. Therefore a further subject matter of the present invention is a method for the detection of an analyte in a sample comprising the steps:

(a) providing a solid phase which comprises a support and at least two spatially separate test areas, the test areas each containing different immobilized analyte-specific receptors,
(b) contacting the sample with the solid phase and at least one free analyte-specific receptor which carries a signal generating group or is capable of binding to a signal generating group, and
(c) detecting the presence or/and the amount of the analyte by determining the signal generating group on the test areas whereby a signal above a predetermined test-area-specific threshold value is classified as positive and a signal below a predetermined test-area-specific threshold value is classified as negative.

The use of predetermined test-area-specific threshold values for each individual test area considerably improves the specificity of detection methods whereas

the sensitivity remains unchanged at a high level. The threshold value or cut-off value is determined from the parameters: sample signal, background of the sample and background of a negative control. A conventional calculation of the cut-off value (COI) is carried out for example according to the formula:

$$COI = signal_{sample} - background_{sample} / n \times background_{negative\ control}$$

A standard value for n is for example 2. The factor n and thus the cut-off value can be increased for particular test areas in which false-positive samples are observed whereby n can be a number between 2 and 100, preferably between 2 and 10.

The threshold values are preferably determined individually for each test area. This means that different threshold values or cut-off values are specified for the various test areas and in particular the threshold for at least two test areas are specified differently. Preferred embodiments of this method utilize the features described above.

The invention is further elucidated by the following examples.

Examples

1. Test for anti-HIV antibodies utilizing microspot technology and several antigen-specific test areas

Microspot is a miniaturized ultasensitive technology which is ideal for the simultaneous determination of

different parameters in a single measurement process. For the determination of anti-HIV antibodies, different HIV detection antigens were each applied individually by means of an inkjet method to a test area (spot) on a polystyrene support in so-called arrays. In this test procedure 30 µl sample diluted in a ratio of 1:1 with sample buffer is pipetted onto the support provided with test areas and incubated for 20 minutes while shaking at room temperature. After aspirating the sample and washing with wash buffer, 30 µl reagent solution 1 which contains a mixture of all digoxigenin-labelled HIV antigens is added and it is again incubated for 20 minutes while shaking at room temperature. After aspirating reagent solution 1 and washing with wash buffer, 30 µl reagent solution 2 containing detection reagent is added. Fluorescent latex particles 100 nm in size which are covalently coated with an anti-digoxigenin antibody are used as a universal detection reagent.

This detection reagent is again incubated for 20 minutes while shaking at room temperature, subsequently aspirated, washed and sucked dry. The test areas are then irradiated with a He-Ne laser at 633 nm wavelength and the fluorescence at 670 nm wavelength is measured with a CCD camera.

The solid phase contains specific test areas containing the following immobilized antigens:

- recombinant p24 polypeptide
- recombinant reverse transcriptase (RT)
- gp41 peptide 1
- gp41 peptide 2

A 50 mM Tris buffer pH 7.6 containing the following additives is used as the sample buffer: 0.05 % Tween 20, 0.5 % bovine serum albumin (BSA), 0.1 % bovine IgG, 0.01 % methylisothiazolone, 3 % peptone.

The sample buffer described above which contains the following test-specific antigens was used as reagent solution 1:

- digoxigenin-labelled, recombinant p24
- digoxigenin-labelled, recombinant reverse transcriptase
- digoxigenin-labelled gp41 peptide 1
- digoxigenin-labelled gp41 peptide 2

50 mM Tris buffer pH 8.0 containing the following additives was used as reagent solution 2: 0.05 % Tween 20, 0.9 % NaCl, 0.5 % BSA, 0.1 % sodium azide and 0.01 % fluorescent-labelled latex particles coated with a monoclonal anti-digoxigenin antibody.

2. Comparison of an anti-HIV antibody test in a microspot format with conventional methods

In this experiment so-called seroconversion samples were measured. These samples are chronological withdrawals from various persons whose serum results converted from HIV-negative to HIV-positive. The more sensitive a test method is, the earlier it can detect a HIV-specific antibody signal. The samples were measured with the method according to the invention (Microspot) and in comparison with a known method (Enzymun® from Boehringer Mannheim). The HIV-specific materials that were used for this were identical in both test systems, which therefore primarily differed only in the separate individual spot analysis. The cut-off indices (cut-off index =

$signal_{sample}$-$signal_{background}$/2x$signal_{negative\ control}$) of the two methods are shown in the following table and additionally compared with Western blot data.

seroconversion panel from the BBI Co.[1]	day of with-drawal	p24	RT	gp41 peptide 1	gp41 peptide 2	Western blot	Enzymun®
R 2nd withdrawal	2	0.0	0.0	0.0	**1.3**	negative	0.5
3rd withdrawal	7	22.2	0.0	0.0	2.4	indifferent	15.4
4th withdrawal	13	17.4	2.6	4.4	36.4	positive	36.0
AB 1st withdrawal	0	0.0	0.0	0.6	0.0	negative	0.3
2nd withdrawal	28	0.0	0.0	0.4	**1.1**	negative	0.7
3rd withdrawal	33	0.0	0.0	5.5	54.4	negative	24.2
4th withdrawal	35	0.8	0.2	6.0	33.2	positive	26.7
5th withdrawal	37	15.2	5.1	4.6	33.0	positive	28.9
AD 5th withdrawal	21	0.0	0.0	0.0	0.0	negative	0.3
6th withdrawal	25	0.2	0.0	**1.6**	**3.7**	positive	0.9
7th withdrawal	28	14.1	0.3	11.1	65.5	positive	24.5
AG 3rd withdrawal	13	0.0	0.0	0.0	0.0	negative	0.4
4th withdrawal	27	0.0	0.0	0.0	**1.1**	negative	0.6
5th withdrawal	34	6.0	5.3	0.0	106.9	positive	8.1
6th withdrawal	50	6.1	5.4	0.0	65.4	positive	3.1
7th withdrawal	78	1.0	6.8	0.0	23.9	positive	1.6
8th withdrawal	163	**1.5**	**5.3**	0.0	**4.9**	positive	0.6
9th withdrawal	194	**2.3**	**2.5**	0.0	**2.8**	positive	0.7
AI 1st withdrawal	0	0.0	0.0	**1.2**	0.1	indifferent	0.8
2nd withdrawal	7	0.6	0.5	54.7	44.9	positive	30.2
3rd withdrawal	11	1.1	0.7	18.8	22.5	positive	30.2

[1]Boston Biomedica Inc.

This comparison shows that the division into individual spots each having optimal antigen concentrations, considerably improves the sensitivity compared to the known tests. Of the 5 seroconversion panels, 7 withdrawals were detected earlier as positive. Depending

on the panel this corresponds to an earlier detection of the HIV infection of 3 to 7 days. Also in comparison to the Western blot, there was a considerable increase of the sensitivity with 6 withdrawels detected earlier.

3. Comparison of a combined determination of HIV p24 antigen and anti-gp41 and anti-RT antibodies in a microspot format with conventional methods

In order to evaluate the sensitivity, so-called seroconversion samples were again measured. They were determined using the method according to the invention (Microspot) and the data obtained were compared with the currently best available anti-HIV tests (cf. see data sheets of the manufacturer of seroconversion panels e.g. BBI Company) or with the Enzymun® combination test from Boehringer Mannheim (combined determination of p24 antigen and anti-HIV antibodies).

The following test areas (individual test spots) (prepared according to example 1) were used for the microspot test format:

- monoclonal anti-p24 antibody A to determine the p24 antigen of HIV subtype B
- monoclonal anti-p24 antibody B to determine the p24 antigen of HIV subtype B and O
- gp41 peptide 1 to determine antibodies against gp41
- gp41 peptide 2 to determine antibodies against gp41
- recombinant reverse transcriptase (RT) to determine antibodies against RT

The HIV-specific materials used in the microspot test were comparable with the materials used in the Enzymun®

test so that the major difference between the Microspot test and Enzymun® method was only the separate individual spot analysis. The cut-off indices (determination see example 2) for the two methods are listed in the following table and additionally compared with the previously most sensitive anti-HIV tests.

seroconversion panel (BBI Co.)[xx]	MAB <p24>A	MAB <p24>B	RT	gp41 peptide 1	gp41 peptide 2	most sensitive <HIV> test	Enzymun combi
Q 1st withdrawal	0.0	0.0	0.0	0.0	0.3	negative	0.30
2nd withdrawal	**24.4**	**48**	0.0	0.0	0.0	negative	0.66
3rd withdrawal	**246**	**435**	0.0	0.0	0.0	negative	**3.47**
4th withdrawal	nd	nd	nd	nd	Nd	positive	**2.30**
W 6th withdrawal	0.1	0.0	0.1	0.0	0.1	negative	0.30
7th withdrawal	0.5	1.1	0.0	0.0	0.1	negative	0.32
8th withdrawal	**5.8**	**14.1**	0.1	0.0	0.1	negative	0.41
9th withdrawal	**529**	**806**	0.0	0.0	0.0	positive	**10.1**
Z 2nd withdrawal	0.0	0.0	0.0	0.0	0.0	negative	0.31
3rd withdrawal	**20**	**25.5**	0.1	0.0	0.1	negative	0.61
4th withdrawal	**226**	**262**	0.0	0.0	0.0	negative	**2.96**
5th withdrawal	0.9	**1.1**	**3.7**	**82.6**	**277**	positive	**18.0**
AD 2nd withdrawal	0.0	0.0	0.0	0.0	0.1	negative	0.30
3rd withdrawal	**2.5**	**6.4**	0.1	0.0	0.1	negative	0.33
4th withdrawal	**96.8**	**200**	0.0	0.0	0.0	negative	**1.5**
5th withdrawal	**663**	**832**	0.0	0.0	0.0	positive	**> 23.3**
6th withdrawal	**549**	**709**	0.6	**2.7**	**2.8**	positive	**> 23.3**
AF 3rd withdrawal	0.1	0.6	0.2	0.0	0.2	negative	0.31
4th withdrawal	0.4	**1.7**	0.0	0.0	0.02	negative	0.30
5th withdrawal	**2.1**	**4.6**	0.02	0.0	0.0	negative	0.34
6th withdrawal	**31.2**	**61.6**	0.1	**2.9**	**204**	positive	**18.0**

xx BBI Boston Biomedica Inc.

This comparison shows that the combined determination of p24 antigen and HIV antibodies by the Microspot test format can be considerably improved compared to conventional methods. Thus the combined Microspot test is many times more sensitive than the Enzymun® "combi-test" in which all antigens and antibodies are present in a mixed form. In the five examined seroconversion panels, nine samples were detected earlier as positive compared to the more sensitive antibody test and six samples were detected earlier as positive compared to the Enzymun® "combi-test".

4. Combined determination of p24 antigen and anti-p24 antibodies by a back titration method

In order to combine the determination of the p24 antigen and of antibodies against p24 in the same array system, a p24 antigen test was carried out in a sandwich format and an anti-p24 antibody test was carried out in a back titration format.

Arrays were prepared comprising the following p24-specific reagents each on individual spots (see example 1):

(a) panel containing p24 antigen and anti-p24 antibody test:

(i) p24 antigen test:

test area 1: monoclonal anti-p24 antibody A Fab' fragment, biotinylated (100 µg/ml)
test area 2: monoclonal anti-p24 antibody B Fab' fragment, biotinylated (100 µg/ml)

(ii) anti-p24 test using the back titration method:

test area 3: biotinylated p24 antigen (0.3 µg/ml)

(b) reference panel comprising anti-p24 antibody test using the bridge method:
- biotinylated p24 antigen (14 µg/ml)

In the test procedure, 30 µl sample diluted in a 1:1 ratio with sample buffer was added by pipette to each panel and incubated for 45 minutes while shaking at 37°C incubation temperature. After aspirating the sample and washing with wash buffer, 30 µl reagent solution 1 which contains a mixture of all digoxigenin-labelled HIV antigens and HIV antibodies was added and incubated for 10 min while shaking at 37°C. The following p24-specific reagents were used:

(a) panel comprising p24 antigen and anti-p24 antibody test:
- monoclonal anti-p24 antibody D $F(ab')_2$ fragment, digoxigenylated (500 ng/ml)
- monoclonal anti-p24 antibody E $F(ab')_2$ fragment, digoxigenylated (500 ng/ml)

(b) reference panel comprising anti-p24 antibody test using the bridge method:
- digoxigenylated p24 antigen (30 ng/ml)

After aspirating reagent solution 1 and washing with wash buffer, 30 µl reagent solution 2 containing detection reagent (see example 1) was added. This detection reagent was incubated for 5 min while shaking at 37°C and was subsequently aspirated, washed and dried.

The test field was irradiated with a He-Ne laser at a wavelength of 633 nm and the fluorescence was measured at a wavelength of 670 nm using a confocal laser

scanner.

11 HIV-negative and 19 HIV-positive samples were measured in comparison using both panels: the cut-off indices (COI) for both test formats are given in the following table.

Sample number	**COI<p24>** back titration*	**COI<p24>** bridge format**
negative control	2412 Cts	93 Cts
positive control	1276 Cts	2451 Cts
negative sample 145	1.39	0.3
negative sample 196	1.54	0.2
negative sample 122	1.43	0.1
negative sample 160	1.41	0.2
negative sample 141	1.28	0.2
negative sample 168	1.58	0.2
negative sample 222	1.38	0.2
negative sample 280	1.42	0.2
negative sample 232	1.32	0.2
negative sample 201	1.54	0.3
negative sample 211	1.33	0.2
positive sample 154	0.31	534
positive sample 132	0.47	537
positive sample 130	0.42	547
positive sample 138	0.46	473
positive sample 163	0.47	591
positive sample 176	0.39	505
positive sample 204	0.39	531
positive sample 167	0.39	588
positive sample 221	0.79	351
positive sample 174	0.30	506
positive sample 285	0.43	506
positive sample 150	0.76	422
positive sample 179	0.58	596
positive sample 236	0.55	573
positive sample 337	0.60	491
positive sample 203	0.35	573
positive sample 147	0.72	610
positive sample 285	0.47	584
positive sample 289	0.30	496

* COI = $signal_{sample}$-$signal_{background}$/0.7x$signal_{negative\ control}$;
COI > 1.0 = negative

** COI = $signal_{sample}$-$signal_{background}$/2x$signal_{negative\ control}$;
COI > 1.0 = positive

All negative as well as all positive samples were correctly detected using the back titration method. The combination of p24 antigen and anti-p24 antibody tests free of mutual interference enables the earlier detection of seroconversion samples and an additional increase in the reliability of false-negative detections.

5. Improvement of the test specificity by test area-specific cut-off calculation

In previously available routine tests, all antigens and antibodies required for the determination are mixed and an optimal cut-off limit is determined for this mixture. This is determined by the unspecific binding of the "worst" test component. In contrast the Microspot technology according to the invention enables a test area-specific cut-off calculation to be carried out which is specific for each test component.

When an identical calculation of the cut-off value (COI = $signal_{sample}$-$background_{sample}$/2 x $background_{negative\ control}$) is used for the individual test areas, it was possible to achieve the following specificity with the HIV combination test (example 3) in 1264 samples:

- p24 antigen: 100 %
- anti-HIV antibody test: 99.52 % (six false-positive determinations)

Since the false-positive determinations only occurred in the two test areas for gp41 peptide 2 and reverse transcriptase, the cut-off limits for these test areas were increased to the following threshold values:

gp41 peptide 2: $COI = signal_{sample} - background_{sample} / 5 \times background_{negative\ control}$

RT: $COI = signal_{sample} - background_{sample} / 3 \times background_{negative\ control}$

In this manner it was possible to improve the specificity of the HIV test from 99.52 % to 99.92 % (only one single false-positive determination). The sensitivity of the test also remained uninfluenced since the cut-off index of the sensitive p24 antigen test was not changed. Thus with an unchanged high sensitivity, it was possible to achieve a considerable improvement of specificity.

Claims

1. Method for the detection of an analyte in a sample comprising the steps:
   (a) providing a solid phase which comprises a non-porous support and at least two spatially separate test areas, the test areas each containing different immobilized analyte-specific receptors,
   (b) contacting the sample with the solid phase and with at least one free analyte-specific receptor which carries a signal generating group or is capable of binding to a signal generating group, and
   (c) detecting the presence or/and the amount of the analyte by determining the signal generating group on the test areas.

2. Method as claimed in claim 1,
   wherein
   the analyte to be detected is a homogeneous or heterogeneous population.

3. Method as claimed in claim 1 or 2,
   wherein
   the analyte is a heterogeneous antibody population, an antigen mixture or a mixture of antigens and antibodies that may be different.

4. Method as claimed in one of the previous claims,
   wherein
   the test areas have a diameter of 0.01 to 1 mm.

5. Method as claimed in one of the previous claims,
wherein
the solid phase is prepared by the separate, direct specific application of the different analyte-specific receptors on the spatially separate test areas.

6. Method as claimed in one of the previous claims,
wherein
the coating on the test areas is in each case composed of a single type of binding molecule.

7. Method as claimed in one of the previous claims,
wherein
a solid phase is used which additionally comprises at least one control area which contains no analyte-specific receptor.

8. Method as claimed in one of the previous claims,
wherein
a universal detection reagent and in particular labelled latex particles are used to detect complexes formed from the analyte and reagents that bind thereto.

9. Solid phase for the detection of an analyte in a sample,
wherein
it comprises a non-porous support and at least two spatially separate test areas, the test areas each containing different reagents which bind specifically to the analyte to be determined.

10. Solid phase as claimed in claim 9,
wherein
the test areas each contain different reagents which bind to different epitopes or/and subtypes of the analyte or/and to different analyte types.

11. Solid phase as claimed in claim 9 or 10,
wherein
the non-porous support is made of polystyrene.

12. Solid phase as claimed in one of the claims 9 to 11,
wherein
the test areas have a diameter of 0.01 to 1 mm.

13. Use of a solid phase as claimed in one of the claims 9 to 12 in an immunoassay.

14. Test kit for the detection of an analyte in a sample comprising a solid phase as claimed in one of the claims 9 to 12 as well as labelled detection reagents.

15. Test kit as claimed in claim 14,
wherein
it contains labelled latex particles as the universal detection reagent.

16. Method for the simultaneous determination of an antigen and of an antibody that is specifically directed against this antigen in a sample comprising the steps:
(a) providing a solid phase on which an immobilized

receptor that can bind to the antigen to be determined is applied in a first test area and an immobilized receptor that can bind to the antibody to be determined is applied in a second test area which is spatially separated therefrom,

(b) contacting the sample with the solid phase and with a free analyte-specific receptor which carries a signal generating group or is capable of binding to a signal generating group and

(c) detecting the presence or/and the amount of the antigen and of the antibody by determining the signal generating group on the solid phase.

17. Method as claimed in claim 16,
wherein
the antigen is detected using a sandwich test.

18. Method as claimed in one of the claims 16 or 17,
wherein
the antibody is detected using a back titration method.

19. Method as claimed in claim 16 or 17,
wherein
the antibody is detected using a bridge method.

20. Method as claimed in one of the claims 16 or 17,
wherein
the antibody is detected using an indirect test format.

21. Method as claimed in one of the claims 16 to 20, **wherein** the coating of the first test area capable of binding is formed from immobilized antibodies which are specific for an epitope of the antigen to be detected.

22. Method as claimed in claim 21, **wherein** antibodies which are specific for different subtypes of the antigen to be detected, are applied in separate test areas.

23. Method as claimed in claim 21 or 22, **wherein** the antibody is selected from viral antibodies, in particular anti-HIV I antibodies, anti-HIV II antibodies, anti-HBV antibodies and anti-HCV antibodies.

24. Method as claimed in one of the claims 16 to 23, **wherein** the coating of the second test area capable of binding is composed of antigens which are specific for the antibodies to be detected.

25. Method as claimed in claim 24, **wherein** the antigens are selected from the group comprising HIV I, HIV II, HBV and HCV.

26. Method as claimed in one of the claims 16 to 25, **wherein**
the antigen to be determined is HIV p24 and the antibody to be determined is anti-p24.

27. Method as claimed in one of the claims 16 to 26, **wherein**
a non-porous solid phase is used.

28. Method as claimed in one of the claims 16 to 27, **wherein**
the detection is carried out using labelled antibodies which are directed against the analyte.

29. Method as claimed in claim 28, **wherein**
the label is selected from fluorescent groups, chemiluminescent groups, radioactive labels, enzyme labels, coloured labels and sol particles.

30. Method as claimed in one of the claims 16 to 29, **wherein**
the detection is carried out using a universal detection reagent in particular labelled latex particles.

31. Method as claimed in one of the claims 16 to 30, **wherein**
the solid phase is prepared by direct, separate application of the specific coatings capable of binding to the individual test areas.

32. Method as claimed in one of the claims 16 to 31,
wherein
the coating on the test areas is in each case composed of a single type of molecule that is capable of binding.

33. Solid phase for the simultaneous determination of an antigen and of an antibody directed specifically against this antigen in a sample comprising at least a first test area and at least a second test area,
wherein
the first test area has a coating that can bind specifically to an antigen and the second test area has a coating which can bind specifically with an antibody directed against the antigen.

34. Solid phase as claimed in claim 33,
wherein
the coatings are homogeneous and each contains only a single type of reagent that is capable of binding.

35. Solid phase as claimed in claim 33 or 34,
wherein
the test areas are applied on a non-porous support.

36. Solid phase as claimed in claim 35,
wherein
the non-porous support is made of polystyrene.

37. Solid phase as claimed in one of the claims 33 to 36,
wherein
the individual test areas have a diameter of 0.01 to 1 mm.

38. Use of a solid phase as claimed in one of the claims 33 to 37 in an immunoassay for the simultaneous detection of an antigen and of an antibody directed specifically against this antigen.

39. Test kit for the simultaneous determination of an antigen and of an antibody directed specifically against this antigen comprising a solid phase as claimed in one of the claims 33 to 37 and labelled detection reagents.

40. Test kit as claimed in claim 39,
wherein
it contains a universal detection reagent.

41. Method for the detection of an analyte in a sample comprising the steps:
(a) providing a solid phase which comprises a support and at least two spatially separate test areas, the test areas each containing different immobilized analyte-specific receptors,
(b) contacting the sample with the solid phase and with at least one free analyte-specific receptor which carries a signal generating group or is capable of binding to a signal generating group, and

(c) detecting the presence or/and the amount of the analyte by determining the signal generating group on the test areas whereby a signal is classified as positive that is above a predetermined test-area-specific threshold value and is classified as negative when it is below a predetermined test-area-specific threshold value.

42. Method as claimed in claim 41,
wherein
the cut-off values are each determined individually for a test area.

43. Method as claimed in claim 41 or 42,
wherein
the cut-off values are set differently for at least 2 test areas.

Abstract

A method for the detection of an analyte in a sample is described comprising the steps:

(a) providing a solid phase comprising a non-porous support and at least two spatially separate test areas, the test areas each containing different immobilized analyte-specific receptors,

(b) contacting the sample with the solid phase and with a second analyte-specific receptor which carries a signal generating group or is capable of binding to a signal generating group and

(c) detecting the presence or/and the amount of the analyte by determining the signal generating group on the solid phase.

Docket No.

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Improvement of binding assays by multiepitope analysis and by combining antigen and antibody determination

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on June 22, 1999 as United States Application No. or PCT International Application Number PCT/EP 99/04310

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
198 27 714.8	DE	June 22, 1998	☐
(Number)	(Country)	(Dav/Month/Year Filed)	
198 38 802.0	DE	August 26. 1998	☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

Marilyn L. Amick, Reg. No. 35,575
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Full name of sole or first inventor KARL Johann
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Assistant Commissioner for Patents
Washington, DC 20231

GENERAL APPOINTMENT OF REPRESENTATIVE FOR U.S. PATENT AND TRADEMARK OFFICE MATTERS

The undersigned applicant or assignee hereby appoints D. Michael Young, Reg. No. 33,819, Richard T. Knauer, Reg. No. 35,575, Brent A. Harris, Reg. No. 39,215, Kenneth J. Waite, Reg. No. 45,189, and Marilyn L. Amick, Reg. No. 30,444 all of Roche Diagnostics Corporation, 9115 Hague Road, P.O. Box 50457, Indianapolis, Indiana 46250, Telephone No. (317) 845-2000, and Jill Lynn Woodburn, Reg. No. 39,874 of The Law Office of Jill L. Woodburn, L.L.C., 6633 Old Stonehouse Drive, Newburgh, Indiana 47630-1785, Telephone No. (812) 842-2660:

to prosecute and transact all business on its behalf before the United States Patent and Trademark Office in connection with any U.S. patent assigned to it and any U.S. patent application filed by it or on its behalf and to receive payments on its behalf.

Signed this 18th **day of** September, **2000 at Mannheim, Germany.**

Roche Diagnostics GmbH

Eichard Jung
Signature

Dr. Michael Jung
Print Name

Director
Position or Title

Roche Diagnostics GmbH

Manfred Weber
Signature

Dr. Manfred Weber
Print Name

Senior Director
Position or Title

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